

**CLAIMS:****1. A method for:**

- (a) differentiating animals and animal products on the basis of breed origin; or
- (b) determining or testing the breed origin of an animal product; or
- (c) validating an animal product;

comprising the steps of:

- (i) providing a sample of the animal product; and
- (ii) analysing the allele(s) of one or more breed determinant genes present in the sample.

**2. The method of claim 1 wherein the breed determinant is a monogenic trait.****3. The method of claim 1 wherein the breed determinant is a polygenic trait.****4. The method of any one of claims 1-3 wherein the overt phenotypic trait is a behavioural or morphological trait.****5. The method of claim 3 or claim 4 wherein the overt phenotypic trait varies qualitatively or quantitatively between breeds.****6. The method of any one of the preceding claims wherein the breed determinant gene analysed in step (ii) is selected from any of:**

- (a) a coat colour gene; and/or
- (b) a coat pattern gene; and/or
- (c) a coat texture gene; and/or
- (d) a coat density gene; and/or
- (e) a coat length gene; and/or
- (f) an ear aspect gene; and/or
- (g) a double muscling gene; and/or
- (h) a horn morphology gene; and/or

- (i) a tusk morphology gene; and/or
- (j) an eye colour gene; and/or
- (k) a plumage gene; and/or
- (l) a beak colour/morphology gene; and/or
- (m) a vocalization (e.g. barking) gene; and/or
- (n) a comb or wattle gene; and/or
- (o) a gene controlling display behaviour.

7. The method of claim 6(a) wherein the coat colour gene is the *KIT* or  *$\alpha$ MSHR* gene (for example, the pig *KIT*  *$\alpha$ MSHR* or gene).

8. The method of any one of the preceding claims wherein the sample is a nucleic acid sample and the analysing step (ii) comprises DNA or RNA analysis.

9. The method of any one of claims 1-7 wherein the sample is a protein sample and the analysing step (ii) comprises protein analysis.

10. A method of determining the coat colour genotype of a pig which comprises:

- (i) obtaining a sample of pig nucleic acid; and
- (ii) analysing the nucleic acid obtained in (i) to determine which allele or alleles of the  *$\alpha$ MSHR* gene is/are present.

11. The method of claim 8 or claim 10 wherein the analysis step (ii) comprises:

- (a) selectively amplifying a specific fragment of nucleic acid (e.g. by PCR); and/or
- (b) testing for the presence of one or more restriction endonuclease sites within the breed determinant gene(s)/ *$\alpha$ MSHR* gene (e.g. restriction fragment length polymorphism (RFLP) analysis); and/or
- (c) determining the nucleotide sequence of all or a portion of the breed determinant gene(s)/ *$\alpha$ MSHR* gene; and/or
- (d) probing the nucleic acid sample with an allele-specific DNA or RNA

probe; and/or

(e) carrying out one or more PCR amplification cycles of the nucleic acid sample using at least one pair of suitable primers and then carrying out RFLP analysis on the amplified nucleic acid so obtained.

12. A method of determining the coat colour genotype of a pig which comprises:

(i) obtaining a sample of pig  $\alpha$ MSHR protein; and

(ii) analysing the protein obtained in step (i) to determine the amino acid sequence at those positions associated with coat colour genotype or the size of the protein

13. The method of claim 9 or claim 12 wherein the analysis step (ii) comprises:

(a) probing the protein sample with an antibody (e.g. a monoclonal antibody) specific for an allele-specific epitope; and/or

(b) electrophoretic analysis; and/or

(c) chromatographic analysis; and/or

(d) amino-acid sequence analysis; and/or

(e) proteolytic cleavage analysis; and/or

(f) epitope mapping and or

(g) translating a copy of the DNA or RNA of the gene produced by PCR or other means in an in-vitro transcription /translation system

14. The method of claim 7 wherein the analysis step (ii) comprises determining the nucleotide sequence of the *KIT* or  $\alpha$ MSHR gene or the amino acid sequence of the *KIT* or  $\alpha$ MSHR protein.

15. The method of claim 7 or claim 14 wherein the analysis step (ii) comprises establishing the presence or absence of at least one nucleotide change in the *KIT* or  $\alpha$ MSHR gene and/or their flanking regions.

16. The method of claim 10 or claim 11 wherein the determination in step (ii)

involves identifying the presence or absence of at least one missense mutation, insertion or deletion in the  $\alpha$ MSHR gene and/or its associated flanking regions.

17. The method of any one of claims 7, 10, 11, 14 and 15 wherein the analysis step (ii) further comprises determining the association between one or more microsatellite or other linked marker alleles linked to the *KIT* or  $\alpha$ MSHR gene and to particular alleles of the *KIT* or  $\alpha$ MSHR gene.

18. The method of claim 17 wherein the analysis step (ii) is based on the identification of microsatellite markers present in the nucleic acid sample.

19. The method of claim 7 wherein the analysis step (ii) comprises:

- (a) determining the association between one or more microsatellite or other linked marker alleles linked to the *KIT* or  $\alpha$ MSHR gene and to particular alleles of the *KIT* or  $\alpha$ MSHR gene;
- (b) determining which microsatellite or other linked marker allele or alleles are present in the nucleic acid sample.

20. A method of determining the coat colour genotype of a pig which comprises:

- (i) determining the association between one or more microsatellite or other linked marker alleles linked to the  $\alpha$ MSHR gene and particular alleles of the  $\alpha$ MSHR gene;
- (ii) obtaining a sample of pig nucleic acid; and
- (iii) analysing the nucleic acid obtained in (ii) to determine which microsatellite or other linked marker allele or alleles are present.

21. The method of any one of claims 7, 10, 11 and 14-20 wherein the analysis step (ii) further comprises the step of determining the genotype of at least one additional locus.

22. The method of claim 21 wherein the additional locus is an additional coat colour locus.
23. The method of claim 22 wherein the additional coat colour locus is the *KIT* gene locus (e.g. the pig *KIT* gene locus).
24. The method of claim 23 wherein the *KIT* gene locus is analysed to determine whether it carries any polymorphism associated with Belt genotype.
25. The method of claim 24 wherein the determination comprises RFLP analysis.
26. A method of determining the coat colour genotype of a pig which comprises:  
    (i) obtaining a sample of pig nucleic acid; and  
    (ii) analysing the nucleic acid obtained in (i) to determine whether the *KIT* gene carries any polymorphism associated with Belt genotype.
27. A method as claimed in claim 26 wherein step (ii) comprises RFLP analysis.
28. A method as claimed in claim 26 or claim 27 wherein a sample of pig genomic DNA is amplified using PCR and a pair of suitable primers.
29. The method of claim 21 wherein the additional locus is a breed determinant gene locus selected from any of those genes specified in claim 6.
30. The method of claim 21 wherein the additional locus is a breed specific marker.
31. The method of claim 30 wherein the breed specific marker is a microsatellite marker.
32. The method of any one of claims 7, 10, 11, 14-23 and 28-31 wherein the analysis

step (ii) comprises PCR using at least one pair of suitable primers.

33. The method of claim 32 wherein the gene is the pig *αMSHR* gene and at least one pair of suitable primers is:

*αMSHR* Forward Primer 1: (5'-TGT AAA ACG ACG GCC AGT RGT GCC TGG AGG TGT-3');

*αMSHR* Reverse Primer 5: (5'-CGC CCA GAT GGC CGC GAT GGA CCG-3'); or

*αMSHR* Forward Primer 2: (5'-CGG CCA TCT GGG CGG GCA GCG TGC-3')

*αMSHR* Reverse Primer 2: (5'-GGA AGG CGT AGA TGA GGG GGT CCA-3'); or

*αMSHR* Forward Primer 3: (5'-GCA CAT CGC CCG GCT CCA CAA GAC-3')

*αMSHR* Reverse Primer 3: (5'-GGG GCA GAG GAC GAC GAG GGA GAG-3').

34. The method of any one of claims 7, 10, 11, 14-23 and 28-33 wherein the analysis step (ii) comprises restriction fragment length polymorphism (RFLP) analysis, for example involving digesting the pig nucleic acid with one or more of the restriction enzymes *Bst*UI, *Hha*I and/or *Bsp*HI.

35. The method of claim 34 wherein the gene is the pig *αMSHR* gene and the analysis involves identification of a polymorphism at nucleotide position 283, 305, 363, 370, 491, 727, 729 1162 or between nucleotide positions 60 and 70 or between nucleotide positions 1005 and 1010 of the sequence of the pig *αMSHR* gene.

36. The method of claim 7 wherein the analysis step (ii) carrying out one or more PCR amplification cycles of the nucleic acid sample using at least one pair of suitable primers and then carrying out RFLP analysis on the amplified nucleic acid so obtained to determine the *KIT* or *αMSHR* genotype of the pig.

37. The method of claim 36 wherein the gene is the pig *αMSHR* gene and the at least one pair of suitable primers is as defined in claim 35.

38. The method of claim 30 or 31 wherein the gene is the pig *KIT* or *αMSHR* gene and the RFLP analysis is as defined in claim 28.

39. The method of any one of claims 1-9, 11, 13-19, 21-25 and 29-38 wherein the animal product is meat (e.g. processed and/or canned meat), egg, egg swab or washing, semen, wool or leather.

40. The method of any one of claims 1-9, 11, 13-19 and 21-39 wherein the sample comprises genomic DNA, RNA, or mitochondrial DNA.

41. The method of any one of claims 1-9, 11, 13-19 and 21-40 wherein the animal is a mammal (e.g. pig, cattle, dog, cat, horse, sheep, rodent or rabbit), fish (e.g. salmon or trout) or bird (chicken or turkey).

42. A kit for:

- (a) differentiating animal products on the basis of breed origin; or
- (b) determining or testing the breed origin of an animal product; or
- (c) validating an animal product;

comprising one or more reagents for analysing the allele(s) of one or more breed determinant genes present in the sample.

43. A kit for determining the coat colour genotype of a pig, comprising one or more reagents for analysing the *αMSHR* genotype of the pig.

44. A kit as claimed in claim 42 or claim 43 which is adapted to be used with a sample of pig genomic DNA.

45. A kit as claimed in any one of claims 42 to 44 comprising one or more reagents for carrying out at least one cycle of PCR together with at least one pair of suitable primers.

46. A kit as claimed in claim 45 wherein the atleast one pair of suitable primers is:

$\alpha$ MSHR Forward Primer 1: (5'-TGT AAA ACG ACG GCC AGT RGT GCC TGG AGG TGT CCA T-3');

$\alpha$ MSHR Reverse Primer 5: (5'-CGC CCA GAT GGC CGC GAT GGA CCG-3'); or

$\alpha$ MSHR Forward Primer 2: (5'-CGG CCA TCT GGG CGG GCA GCG TGC-3')

$\alpha$ MSHR Reverse Primer 2: (5'-GGA AGG CGT AGA TGA GGG GGT CCA-3'); or

$\alpha$ MSHR Forward Primer 3: (5'-GCA CAT CGC CCG GCT CCA CAA GAC-3')

$\alpha$ MSHR Reverse Primer 3: (5'-GGG GCA GAG GAC GAC GAG GGA GAG-3').

47. A kit as claimed in any one of claims 42 to 46 which comprises one or more reagents for RFLP analysis of pig nucleic acid.